

What is claimed is:

1. A method for detecting or quantifying a known target polynucleotide having a known nucleotide sequence comprising:

(a) hybridizing a first primer to the known target polynucleotide and
5 extending the primer using a non-terminator nucleotide mixture formulated to produce equal length primer extension products;

(b) hybridizing the equal length extension products to a second primer;

(c) producing extension products from the second primer; and

(d) detecting the extension products from the second primer.

10 2. The method of claim 1, wherein the extension products of the first primer comprise a primer portion and an extended portion.

3. The method of claim 2, wherein the second primer hybridizes to the extended portion of the extension products of the first primer.

15 4. The method of claim 2, wherein the second primer is not complementary to the first primer.

5. The method of claim 1, wherein the amount of detectable extension product correlates to the amount of target polynucleotide.

6. The method of claim 1, wherein the hybridization of the first and second primers occurs under high stringency.

20 7. The method of claim 1, wherein the extension products from the second primer are detected using fluorescence spectroscopy or mass spectroscopy.

8. The method of claim 1, wherein the extension products from the second primer comprise a detectable label.

25 9. The method of claim 8, wherein the labeled comprises an epitope, fluorophore, metal particle, enzyme, carbohydrate, polypeptide, radioactive isotope, dye, biotin, or digitonin.

10. The method of claim 1, wherein the primer comprises deoxyribonucleic acid, ribonucleic acid, or a combination thereof.

11. The method of claim 1, wherein the nucleic acid of interest comprises deoxyribonucleic acid, ribonucleic acid, or a combination thereof.

12. The method of claim 1, wherein the extension products are enzymatically produced.

13. The method of claim 12, wherein the enzyme is template-dependent.

14. The method of claim 13, wherein the template-dependent enzyme is DNA
5 polymerase, RNA polymerase or reverse transcriptase, or a combination thereof.

15. The method of claim 1, wherein the target polynucleotide is synthesized enzymatically *in vivo*, *in vitro*, or synthesized non-enzymatically.

16. The method of claim 1, wherein the target polynucleotide is synthesized by polymerase chain reaction.

10 17. The method of claim 1, wherein the target polynucleotide comprises genomic DNA from an organism, RNA transcripts thereof, or cDNA prepared from RNA transcripts thereof.

18. The method of claim 17, wherein the organism is a plant, microorganism, bacteria, virus.

15 19. The method of claim 17, wherein the organism is a vertebrate or invertebrate.

20. The method of claim 17, wherein the organism is a mammal.

21. The method of claim 20, wherein the organism is a human being.

22. The method of claim 1, wherein an amplification step is performed on the target polynucleotide.

20 23. The method of claim 1, wherein the first primer comprises one or more moieties that permit affinity separation of the primer from unincorporated reagent and/or the polynucleotide of interest.

24. The method of claim 1, wherein the second primer comprises one or more moieties that allows immobilization of the second primer onto a solid support to produce an
25 immobilized second primer sequence.

25. The method of any one of claims 23 or 24, wherein the moieties comprises a biotin, digitonin, a phosphate group, or amine group.

26. The method of claim 1, wherein the second primer is synthesized directly on a solid support to produce an immobilized second primer sequence.

27. The method of claim 26, wherein the synthesis is accomplished enzymatically, chemically, or physically.

28. The method of claim 1, the first or second primer is immobilized onto a solid support to produce an immobilized target nucleic acid sequence.

5 29. The method of claim 28, wherein the first or second primer can be cleaved from the solid support by a chemical, enzymatic or physical process.

30. The method of claim 28, wherein immobilization is accomplished via a photocleavable bond.

10 31. The method of claim 28, wherein the solid support comprises beads, flat surfaces, chips, capillaries, pins, combs or wafers.

32. The method of claim 28, wherein the immobilization of the first primer is accomplished by hybridization between a complementary capture nucleic acid molecule, which has been previously immobilized to a solid support.

15 33. The method of claim 28, wherein said immobilization is accomplished via direct bonding between the solid support and a portion of the nucleic acid molecule, which is distinct from the target nucleic acid sequence.

34. A kit for quantification of a target nucleic acid comprising:

(a) a first primer complementary to a polynucleotide sequence of the target nucleic acid;

20 (b) a second primer complementary to an extension product formed from the first primer;

(c) one or more enzymes for performing a primer extension reaction; and

(e) a non-terminator nucleotide mixture formulated to produce equal length primer extension products.

25 35. The kit of claim 34, wherein at least one non-terminator nucleotide of the non-terminator nucleotide mixture comprises a detectable label.

36. The kit of claim 34, wherein the one or more enzymes comprise a polymerase or reverse transcriptase.

37. The kit of claim 34, where in said non-terminator nucleotide mixture is formulated to prevent at least one canonical Watson-Crick base pair during the extension reaction.

38. The kit of claim 34, wherein the enzyme is a template dependent enzyme.

5 39. The kit of claim 38, wherein the template dependent enzyme is DNA polymerase, RNA polymerase, or reverse transcriptase.

40. The kit of claim 34, wherein the second primer is immobilized on a solid support.

41. The kit of claim 35, wherein the detectable label comprises an enzyme, protein moieties, radioactive isotope, dye, fluorescent moieties, biotin, or digitonin.

10 42. The kit of claim 34, further comprising a buffer solution.

43. The kit of claim 34, wherein the non-terminator nucleotide mixture includes nucleotides consisting of X, Y, and Z, wherein X and Y are different purine non-terminator nucleotides, and Z is a pyrimidine non-terminator nucleotide; or X and Y are different pyrimidine non-terminator nucleotides, and Z is a purine non-terminator nucleotide.

15 44. A method of detecting a target polynucleotide comprising:

(a) hybridizing a first primer to the target polynucleotide;

(b) forming equal length primer extension products using a nucleotides consisting of X, Y, and Z, wherein X and Y are different purine non-terminator nucleotides, and Z is a pyrimidine non-terminator nucleotide; or X and Y are different pyrimidine non-terminator nucleotides, and Z is a purine non-terminator nucleotide;

20 nucleotides, and Z is a purine non-terminator nucleotide;

(c) hybridizing a portion of the extension products of (b) to a second primer;

(d) extending the second primer with at least one nucleotide having a detectable marker using a portion of the equal length extension products of (b) as a template; and

25 (e) correlating the amount of detectable marker in the extension products of (d) with the amount of target polynucleotide.

45. The method of claim 44, wherein the second primer hybridizes to the non-primer portion of the extension product of (b).

46. The method of claim 44, wherein a primer portion of the extension products of (b) serves as a template strand for extending the second primer

47. A method for diagnosing a host, comprising:

(a) obtaining from the host a sample comprising a polynucleotide;
(b) contacting the sample with a first primer, the first primer comprising a nucleotide sequence complementary to a portion of a known target polynucleotide;

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(c) extending the primer using a non-terminator nucleotide mixture formulated to produce equal length primer extension products;

(d) hybridizing the equal length extension products to a second primer;

(e) producing extension products from the second primer; and

(f) detecting the extension products from the second primer, wherein the

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detection of an extension product from the second primer is indicative of a pathology or predisposition to a pathology of the host.

48. The method of claim 47, wherein the known target polynucleotide is an oncogene or variant thereof.